

## Antennal resolution of pulsed pheromone plumes in three moth species

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### Abstract

Male antennae of *Cadra cautella*, *Pectinophora gossypiella*, and *Spodoptera exigua* were presented with 20-ms-duration pulses of their two-component pheromone at rates of 1 to 33 Hz. Fourier analyses of electroantennograms resolved the temporal structure of trains of pheromone filaments delivered at up to 33 Hz for *C. cautella* and *S. exigua* and 25 Hz for *P. gossypiella*. Pheromone components tested separately for each species were generally equivalent in filament resolution to complete blends. Ambient temperatures of 18, 23 and 28°C affected filament resolution only slightly, with poorer ability to discriminate rapidly pulsed signals at 18°C. The question of how, or indeed if, such frequencies are conserved beyond the peripheral nervous system, remains.

**Keywords:** *Spodoptera exigua*; *Cadra cautella*; *Pectinophora gossypiella*; pheromone plume; electroantennogram; temporal resolution.

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## 1. Introduction

The sinuous and filamentous structure of pheromone plumes is caused mainly by turbulent diffusion. An insect flying along such plumes encounters pheromone as a series of bursts of variable concentration and spacing (Murlis and Jones, 1981; Murlis et al., 2000). Baker (1990) proposed that progress upwind toward the source was modulated in flying moths by 'moment-to-moment' interception of pheromone filaments that make-up the plume. Upon contact with a single filament, male moths briefly surge upwind (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994), and if no more filaments are intercepted, moths adopt a more crosswind heading (Mafra-Neto and Cardé, 1996; Quero et al., 2001). Moths flying along pulsed plumes that are mechanically generated head more directly upwind when presented with a 4 Hz pulse rate than with lower rates (Mafra-Neto and Cardé, 1995b; Vickers and Baker, 1996). During upwind flight, a moth encounters filaments of pheromone at a rate that depends not only on the spacing between filaments but also on the moth's upwind velocity. *Cadra cautella* males flying along pulsed pheromone plumes at generated frequencies between 1.7 and 5 Hz in wind speeds between 20 and 80 cm s<sup>-1</sup> intercepted filaments at rates that ranged from 1.3 to 3-fold higher than the frequency of pulse generation (Mafra-Neto and Cardé, 1998). If the frequency of pheromone filament interception exceeds the response rate of the insect's neural circuitry, however, it is likely that the flickering signal would be perceived as a continuous or 'fused' signal.

Studies with the tortricids *Adoxophyes orana* (Kennedy et al., 1981) and *Grapholita molesta* (Willis and Baker, 1984; Baker et al., 1985) in homogeneous pheromone clouds revealed that fluctuating pheromone stimulation was required for upwind flight. However, the males of the pyralid *C. cautella* fly upwind both in homogeneous clouds of pheromone (Justus and Cardé, 2002) and along pheromone plumes pulsed at rates as high as 25 Hz (Justus et al., 2002a). To assess the ability of moths from different lineages to resolve flickering pheromone plumes at the antennal level, we tested three moth species from different families: the beet armyworm, *Spodoptera exigua* (Noctuidae), the almond moth, *C. cautella* (Pyralidae), and the pink bollworm, *Pectinophora gossypiella* (Gelechiidae).

To ascertain the flicker rate that potentially could be discriminated, we delivered trains of pheromone pulses at 1 to 33 Hz to antennae positioned downwind of the dispenser pipette. Because individual components of the pheromone blend may affect receptor cells differently (Rumbo and Kaissling, 1989), the

effect of blend completeness on the antennal resolution of filaments was investigated by stimulating antennae with individual components and with the pheromone blend.

There also is evidence that temperature modulates electrophysiological and behavioral responses of male moths to pheromone. At 23°C, antennal neurons of *G. molesta* sensitive to the main component of its pheromone responded with bursts of action potentials that corresponded to trains of slowly pulsed pheromone (3 Hz), whereas at 17°C antennal neurons produced fewer action potentials per second and bursts were less distinguishable (Baker et al., 1988). *G. molesta* males flying at 20°C also showed a significantly reduced ability to sustain upwind flight in certain blends and dosages of pheromone in comparison to males flying at 26°C (Linn et al., 1988). In wind tunnel experiments, *Lymantria dispar* flew along pheromone plumes at higher airspeeds and ground speeds at 26°C compared to 20°C (Charlton et al., 1993). To assess if temperature affects the ability of moth antennae to resolve flickering signals, experiments were conducted with all three species at 18, 23, and 28°C.

In many moths, seasonal and diel fluctuations in ambient temperature modulate the timing of pheromone emission and male attraction. Typically with the generally nocturnal species, cooler temperatures advance sexual communication to earlier in the scotophase and sometimes even into photophase. Attraction in *P. gossypiella*, for example, occurs in early evening in spring and fall flights, and after midnight in summer (Lingren et al., 1989). Attraction to synthetic pheromone occurs at temperatures from 12 to 30°C (Lingren et al., 1989), although catch is reduced below 18°C (Adams et al., 1995). For *S. exigua*, a minimum flight temperature of 15.6°C has been reported (Hogg and Gutierrez, 1980). The three ambient temperatures tested in our trials encompass a range that our test species would encounter in the field.

## 2. Materials and methods

### 2.1. Insects

*S. exigua* was reared on an artificial diet based on lima beans (modified from Shorey and Hale, 1965). Pupae were sexed and males were placed singly in 4 cm high by 4 cm diameter plastic containers until emergence. *C. cautella* was reared on a diet consisting of poultry laying mash, rolled oats, brewer's yeast and

glycerin (see Mafra-Neto and Cardé, 1995a). Larvae in their last instar were sexed and males were put in 18 by 12 by 7 cm plastic containers until pupation, when they were transferred singly to tubes of 5 cm long and 1 cm diameter until emergence. *P. gossypiella* pupae were obtained from USDA/APHIS. Pupae were sexed and males were placed singly in tubes of 5 cm long and 1 cm diameter until emergence.

## 2.2. Synthetic Pheromone

Synthetic pheromone was formulated gravimetrically in hexane solutions of  $10 \mu\text{g } \mu\text{l}^{-1}$  that were serially diluted to obtain pheromone blend and single component concentrations of 10 and  $1 \text{ ng } \mu\text{l}^{-1}$ . The pheromone blend for *S. exigua* was a 10:1 mixture of (Z,E)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac) and (Z)-9-tetradecenol (Z9-14:OH) (purities of 93.9% and 93.6% respectively; Bedoukian Research, Danbury, CT, USA), for *C. cautella* a 10:1 mixture of Z9,E12-14:Ac and (Z)-9-tetradecenyl acetate (Z9-14:Ac) (purities of 93.9 and 96.3% respectively; Bedoukian) and for *P. gossypiella* a 56:44 mixture of (Z,Z)-7,11-hexadecadienyl acetate (Z7,Z11-16:Ac) and (Z,E)-7,11-hexadecadienyl acetate (Z7,E11-16:Ac) (Bedoukian, both were > 99% free of geometric isomers).

## 2.3. Wind Tunnel

Experiments were conducted in a wind tunnel 3 m long by 1 m wide with a floor made of Plexiglas<sup>®</sup> and with a Vivac<sup>®</sup> sheet bent in semi-cylindrical shape that covers its working space (see Justus et al., 2002a,b). Wind speed was set at  $50 \text{ cm s}^{-1}$ .

## 2.4. Odor-delivery system

Using a Stimulus Flow Controller (SFC-2, Syntech, Hilversum, The Netherlands), sequences of 20-ms-duration pulses were delivered at a flow rate of  $2.5 \text{ ml s}^{-1}$  from a Pasteur pipette containing a 7.5 mm-diameter filter paper disk impregnated with the stimulus. Except for temperature trials, the filter paper disk was positioned in the upper part of the pipette at ca. 1.5 cm from the outlet to minimize adsorption of pheromone to the glass surface of the pipette. The tip of the pipette was bent at  $\sim 135^\circ$  angle and faced upwind, thereby

producing discrete puffs of pheromone even at high pulsing frequencies (Justus et al., 2002a). The pipette outlet was positioned 40 cm from the upwind end of the wind tunnel and 30 cm above its floor.

In experiments performed at 18, 23 and 28°C, we monitored temperature in the wind tunnel with a thermocouple probe (type T, Omega<sup>®</sup>, Stamford, CT, USA) placed 3 cm above and 15 cm downwind of the antennal preparation. To ensure that the rate of pheromone released from the pipette into the wind tunnel remained constant, the dispenser temperature was held at 23±0.5°C. This was achieved by placing the pipette in an insulated glass condenser connected to a circulating pump (Isotemp 1006S, Fisher Scientific, Pittsburgh, PA, USA) that maintained a flow of water with polyethylene glycol at a constant temperature. The filter paper disk was positioned 3 cm from the pipette outlet and a thermocouple probe inside the tubing monitored the temperature of the air flowing over the disk.

### *2.5. Electroantennogram*

The electroantennogram (EAG) probe (PRG-2, Syntech) was mounted on a stand inside the wind tunnel, 10 cm downwind from the dispenser pipette, except for the temperature experiments in which the probe was 5 cm downwind. The stand was grounded to a layer of aluminum foil that covered the inner surface of the wind tunnel 50 cm upwind and downwind of the EAG probe. Pulsed trains of TiCl<sub>4</sub> 'smoke' were used before mounting each antenna so that it could be positioned precisely within the plume's boundaries.

Males 2-3 days old were decapitated and an antenna was excised with a razor blade. After removing the most distal segments, the antenna was mounted between the recording and reference electrodes using electrode gel (Spectra 360, Parker Laboratories Inc., NJ, USA) with the ventral side of the antenna facing upwind. EAG signals were acquired through a PC interface board (IDAC-02, Syntech) and Autospike32 (Syntech) software.

### *2.6. Experiments*

Pulsing regime: Pulsed stimulations of 1, 2, 3, 4, 5, 10, 13, 17, 25 and 33 Hz were recorded for *ca.* 5 s, with 30 s between recordings of different pulsing frequencies. In all cases, each pulse was of 20 ms duration. Ten antennae were tested for each treatment, alternating series of increasing and decreasing pulsing

frequencies. After the series of pulsed stimulations, a continuous 5 s stimulation was recorded for each antenna.

**Pheromone doses:** Doses of 10 and 100 ng impregnated on a filter paper disk were tested for all three species. Pheromone components were also tested separately, maintaining their dose in an amount relative to 100 ng of pheromone blend. Thus, we tested doses of 100 ng of Z9,E12-14:Ac and 10 ng of Z9-14:OH for *S. exigua*, 100 ng of Z9,E12-14:Ac and 10 ng of Z9-14:Ac for *C. cautella*, and 56 ng of Z7,Z11-16:Ac and 44 ng of Z7,E11-16:Ac for *P. gossypiella*. With *S. exigua* and *C. cautella*, the minor components of the pheromone blend (Z9-14:OH and Z9-14:Ac, respectively) were also tested at 100 ng. Five additional recordings at 1 Hz were made for *C. cautella* with doses of 200 and 50 ng of pheromone blend to establish possible correlations between dose and EAG peak characteristics.

**Temperature:** Experiments were conducted with all three species at ambient temperatures of 18, 23 and 28°C ( $\pm 0.5^\circ\text{C}$ ). The stimulus consisted of 100 ng of the respective pheromone blend on a filter paper disk and delivered in 20 ms puffs pulsed at 0.3, 1, 3, 5 and 10 Hz. Ten replicates were made for each treatment, alternating the order of presentation between increasing and decreasing pulsing frequencies.

## 2.7. Data analysis

A custom computer program in Visual Basic® was used to analyze EAG signals as follows:

**Pulsing regime:** For all frequencies of 1 to 25 Hz, percent of return to baseline between peaks was calculated using the peak amplitude and depolarization value at the onset of the following peak (i.e.,  $\text{Percent Return}_1 = (1 - (\text{Value}_1 / \text{Value}_2)) * 100$ ; Fig. 1A). Means were calculated for each replicate. Second-order means were compared among frequencies within species using ANOVA and Tukey HSD for unequal sample sizes (Spjotvoll/Stoline) post-hoc test.

**Periodicity analysis:** Starting at the onset of stimulation, 3 s duration portions of EAG recordings of stimulation frequencies between 10 and 33 Hz were exported to Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA) for spectral frequency analysis (Single Series Fourier Analysis). This procedure uses the Fast Fourier Transforms algorithm to convert a time series into a frequency spectrum that can be plotted as a periodogram. Periodogram values of every recording were averaged with the corresponding values from all antennal preparations stimulated at the same pulse frequency within the same treatment, and the resulting graphs for

each stimulation frequency were merged into a single graph. In some cases, in addition to frequencies detected from periodic fluctuations in the EAG recordings, peaks corresponding to harmonics of these frequencies also appeared in the periodogram. Such peaks were removed for clarity.

**Pheromone doses:** Values of depolarization amplitude (peak amplitude), depolarization time, and repolarization time to two-thirds peak amplitude ( $t = \text{time}_{1/3} - \text{time}_1$ ; Fig. 1B), were obtained using the first peak of each 1 Hz recording. Means among pheromone treatments (doses of complete blend and single components) within species were compared using ANOVA and Tukey HSD for unequal sample sizes (Spjotvoll/Stoline) post-hoc test. Comparisons among species were made for recordings with 100 ng of the respective pheromone blend using ANOVA and Tukey HSD post-hoc Test. Data from *C. cautella* recordings after stimulation with 200, 100, 50 and 10 ng of pheromone blend (5 replicates for each dose) were used to perform a correlation analysis (Pearson's  $r$ ) between amplitude of depolarization and both depolarization and repolarization to two-thirds of peak amplitude times.

**Temperature:** Values of depolarization time, repolarization time to two-thirds peak amplitude and peak amplitude were analyzed as in pheromone doses. Second-order means of percent return to baseline for frequencies between 0.3 and 10 Hz were obtained as in pulsing regime experiments, and for each species, compared among temperatures within each frequency using ANOVA and Tukey HSD post-hoc test.

### 3. Results

Electroantennographic recordings for all three species were obtained at stimulation rates from 1 to 33 Hz (Fig. 2; frequencies of 2, 3 and 4 Hz not included). Because pulses are discrete (Justus et al., 2002b), an 'ideal' sensor would recover to baseline between peaks. However, in the EAG recordings presented here, as the frequency of pulses increased, peaks began to 'fuse'; that is, they did not return to baseline before the next depolarization (Fig. 2).

Although peaks are fused in EAG recordings obtained at the highest pulsing rates evaluated, Fourier analysis resolved pulsed stimulations at frequencies as high as 25 Hz for *P. gossypiella* and 33 Hz for *S. exigua* and *C. cautella*. Individual pheromone components produced the same level of temporal resolution as

the two-component blends. With 100 ng of each component as a stimulus, Fourier analysis resolved the same pulsing frequencies as 100 ng of the pheromone blend (data not shown).

For *S. exigua*, full recovery of the EAG signal between stimulations occurred up to 5 Hz (Fig. 4). Percent return to baseline by antennae of the other two species was much lower at these frequencies, and showed a decrease in recovery even at 2 Hz. At frequencies as high as 10 Hz, *S. exigua* still showed a return to baseline of 61.1% ( $\pm 12.6$  SD), whereas at this pulsing rate *C. cautella* and *P. gossypiella* recovered to 21.7% ( $\pm 6.5$  SD) and 16.9% ( $\pm 1.8$  SD) of baseline, respectively.

Differences in the shape of the EAG peaks among species (Fig. 2) were confirmed by analyses of depolarization times, repolarization times to two-thirds of the peak amplitude, and depolarization amplitudes (Fig. 5). There were no significant differences in peak amplitude among species. However, repolarization time was significantly shorter for *C. cautella* than for *P. gossypiella*, and both depolarization and repolarization times were shortest for *S. exigua*.

Shorter repolarization times together with lower depolarization amplitudes were also observed in *S. exigua* and *C. cautella* when the amount of pheromone blend was reduced from 100 ng to 10 ng ( $P < 0.05$ ; Fig. 5). Furthermore, there were positive correlations for *C. cautella* between peak amplitude (-mV) and depolarization time ( $P < 0.05$ ; Pearson's  $r = 0.54$ ) and between peak amplitude and repolarization time to two-thirds of peak amplitude (Pearson's  $r = 0.92$ ). Despite a significant reduction in peak amplitude, repolarization times for *P. gossypiella* were not significantly different (Fig. 5).

Differences in antennal response to individual pheromone components were found in *S. exigua* and *C. cautella*. In these species, the main component and the pheromone blend had a longer repolarization time than the minor component alone. However, peak amplitudes were also much higher for the blend and main component than for the minor component.

Changes in ambient temperature modulated the EAG response of all three species similarly. In all cases, EAG signals showed poorer recovery between pulses when temperatures were lower (Fig. 6). Peak amplitude was not affected by temperature except for *P. gossypiella*, which showed lower depolarization amplitude at 18°C. Depolarization and repolarization times were briefest at 28°C, and increased as the temperature declined (Fig. 7).



#### 4. Discussion

Odor plumes in wind that are generated from a point source of pheromone, are intermittent owing to the effects of atmospheric turbulence (Murlis et al., 2000). Some male moths require such a flickering signal to maintain their upwind flight toward a pheromone source (Kennedy et al., 1981; Willis and Baker, 1984; Baker et al., 1985). Therefore, the assessment of the sensory system's ability to resolve a rapidly flickering pheromone plume is of importance in understanding the relationships between male moth orientation maneuvers and the plume's fine-scale features.

Lemon and Getz (1997) reported that antennae of the cockroach, *Periplaneta americana*, resolved pulses of 'general' odors (e.g., coconut oil) at rates up to 20 Hz. However, some projection neurons (PN) in the same species did not preserve the temporal structure of the stimulus detected at the antennal level, and it was suggested that the PNs might encode chemical composition rather than the temporal dynamics of the stimulus (Lemon and Getz, 2000).

In contrast, pheromone-specific sensory cells of male moths are tuned to the components of their pheromone blend. Because the perception of intermittencies within the pheromone plume seems to be important to keeping moths of some species flying upwind (Kennedy et al., 1981; Willis and Baker, 1984; Baker et al., 1985), it is probable the temporal structure of the stimulus is conserved at a central level. Some PNs from the macroglomerular complex of *Manduca sexta* males discriminate both duration and interpulse intervals of pheromone arriving at the antenna at *ca.* 10 Hz (Christensen and Hildebrand, 1997). Although there is no evidence that pulse trains higher than 10 Hz can be resolved by PNs, simultaneous intracellular and EAG recordings on *Heliothis virescens* indicate that the activity of at least some olfactory PNs is time-locked to the stimulus dynamics (Vickers et al., 2001). It is possible, therefore, that high frequency fluctuations of stimulus concentration resolved peripherally are encoded at more central processing levels.

At the peripheral level, resolution of discrete pheromone puffs pulsed at moderate frequencies has been reported in two species. Single sensillum recordings showed that *M. sexta* receptor cells followed 20 ms pulses at a pulsing rate up to 3 Hz (Marion-Poll and Tobin, 1992). Likewise, Rumbo and Kaissling (1989) reported that two of the three sensory cell types described in *Antheraea polyphemus* followed 20 ms stimulations pulsed at frequencies up to 5 Hz. In our experiments with moths of three taxonomic families, Fourier analysis resolved periodic fluctuations in EAG recordings with stimulus intermittencies as high as 25

Hz. Each antenna was held in the center of the plume perpendicular to airflow, with trichodeal sensilla projecting into the flow, an arrangement most apt to provide a simultaneous arrival of pheromone at all antennal segments. However, at the highest frequencies of pheromone delivery, EAG peaks were partially or even mostly fused, because the repolarization between responses to pulsed stimulations was small in relation to the overall EAG depolarization (Fig. 2). Although Fourier analysis demonstrates resolution of signals peripherally, it is unknown if the periodicity of such signals is resolved and encoded at a more central level.

Depolarization and repolarization times varied among species presented with 100 ng of their pheromone blend. As well, depolarization time and repolarization time to two-thirds peak amplitude showed a strong correlation with peak amplitude within species. However, because amplitudes of depolarization were similar between species, differences in repolarization time were presumably because of differences in rates of repolarization. Faster recovery rates allowed *S. exigua* to better resolve pulses at higher frequencies than *C. cautella* and *P. gossypiella*.

Individual components of the pheromone blend also can elicit different temporal responses for their pheromone receptor cells (Rumbo and Kaissling, 1989). In *M. sexta*, some PNs encode the temporal pattern of the stimulus with improved fidelity if presented with the pheromone blend rather than with just the 'main' component (Christensen and Hildebrand, 1997). A parallel study of *Agrotis segetum*, however, showed that interactions between different pheromone components did not improve the pulse-resolving capability of antennal lobe neurons (Lei and Hansson, 1999). Although an effect at a more central processing level in the three species studied here cannot be ruled out, EAG recordings showed comparable resolution abilities in response to either the full pheromone blend or individual components.

Differences in EAG peak shape found at ambient temperatures of 18, 23 and 28°C, and a diminution of the recovery of electrophysiological signal at modest rates of 5 Hz, are of uncertain behavioral consequence. It would appear that filaments of pheromone encountered at rates above 3 to 5 Hz would be more poorly resolved at 18°C. In principle at lower temperatures this should result in the peripheral signal 'fusing' at a somewhat lower rate of filament encounter. In the case of *C. cautella*, such a fused signal might cause a moth flying along a turbulent plume, on average, to head somewhat more toward crosswind at the lower temperature, based on how rates of filament encounter generally alter the flight track of this moth (Mafra-

Neto and Cardé, 1995b). But this species also heads upwind in a homogeneous cloud of pheromone (Justus and Cardé, 2002), in which case there is no internal filamentous structure.

In nature, filaments of pheromone would impinge on a moth in irregular bursts (Murlis et al., 2000), in which the rate of filament encounter varies considerably. The small magnitude of change in filament resolution seen among the three ambient temperatures tested might be of little consequence in orientation to natural plumes in which the rate of filament encounter is not based on the kind of fixed frequencies evaluated here. Furthermore, the performance of whole animal preparations, versus the excised antennae used in this study, at these temperature regimes is undetermined. If there is any disparity, it is likely that temperature would have less of an effect on whole animal preparations. We consider the ambient temperatures employed in these studies to be unlikely to have a major effect on how these three moth species perceive their moment-to-moment contact with filaments of pheromone. Determination of such a temperature-modulated effect on filament recognition in a freely flying moth will be difficult, given that ambient temperature can alter a moth's airspeed, upwind heading, and, likely, its threshold of pheromone perception (Charlton et al., 1993).

The issue of how rates of filament encounter are preserved and interpreted at a more central processing level (e.g., in the PNs) remains unsettled. The Fourier analyses of EAG responses likely reflect the maximum capability of antennal sensing of filament frequency. In *C. cautella*, males are capable of flying upwind along plumes pulsed at high frequency (25 Hz) (Justus et al., 2002a) and within homogeneous clouds of pheromone for ca. 2 seconds (Justus and Cardé, 2002). In two tortricid moths (Kennedy et al., 1981; Baker et al., 1985) and a gelechiid moth (Justus and Cardé, 2002), however, homogeneous clouds of pheromone inhibited upwind flight, implying in these species that signal fusion at high rates of filament encounter governs inhibition of upwind progress. The approaches used in the current EAG measurements need to be followed by explorations of how well such pulsed signals are conserved centrally.

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### Figure captions

**Fig. 1.** Parameters considered in the analysis of EAG recordings from pulsed trains of pheromone (A) where  $\text{Percent Return}_1 = (1 - (\text{Value}_{1'} / \text{Value}_1)) * 100$ , and single pulses (B) where  $r = \text{time}_{1'} - \text{time}_1$ . **d:** depolarization time, **r:** repolarization time to two-thirds of peak amplitude.

**Fig. 2.** EAG recordings from antennae of male *S. exigua*, *C. cautella* and *P. gossypiella* male antennae placed 10 cm downwind from the odor source in response to 20 ms pulses of pheromone blend (100 ng) delivered at different rates (1, 5, 10, 13, 17, 25 and 33 Hz) and to continuous stimulation (Cont.).

**Fig. 3.** Combined periodogram plots after Fourier analysis of EAG recordings on *S. exigua*, *C. cautella* and *P. gossypiella*. Stimulus was mechanically pulsed at frequencies of 10, 13, 17, 25 and 33 Hz and continuous stimulation (gray line). Stimulus was 100 ng of the respective pheromone blend and the duration of every pulse was 20 ms. Results based on 10 replicates of recordings of 3 s duration.

**Fig. 4.** Return to baseline (percent  $\pm$  SD) of the EAG signal between pulses, at frequencies of 1 to 25 Hz. Different letters denote significant differences among frequencies within each species (ANOVA and Tukey HSD for unequal sample sizes (Spjotvoll/Stoline) post-hoc Test;  $\alpha=0.05$ ,  $P < 0.05$ ). Six to ten replicates per treatment.

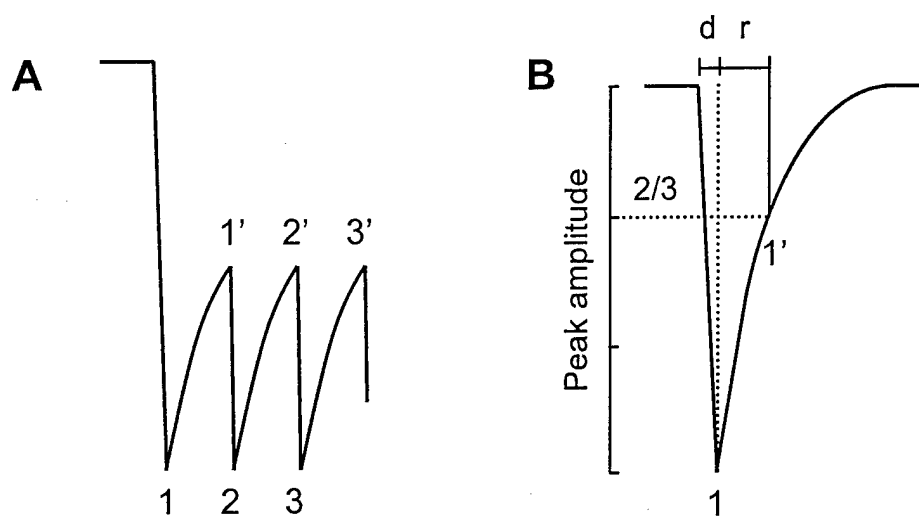
**Fig. 5.** Peak characteristics calculated from EAG recordings of *S. exigua*, *C. cautella* and *P. gossypiella*. Responses ( $\pm$  SD) were averaged from 6 replicates for 10 ng pheromone blend experiments in all three species, 9 replicates for 100 ng Z9-14:OH in *S. exigua* and 10 replicates in all other treatments. Repolarization time was calculated from the highest depolarization value to two-thirds of the peak amplitude. Different lower case letters denote significant differences within the same species (ANOVA and Tukey HSD for unequal sample sizes (Spjotvoll/Stoline) post-hoc Test;  $\alpha=0.05$ ,  $P < 0.05$ ). Asterisks denote significant differences for 100 ng of pheromone blend among species (ANOVA and Tukey HSD post-hoc Test;  $\alpha=0.05$ ,  $P < 0.05$ ).

**Fig. 6.** Return to baseline of the EAG signal (percent  $\pm$  SD) between stimuli pulsed at frequencies of 0.3 to 10 Hz at room temperatures of 18, 23 and 28°C. Results are the average from 10 replicates of each treatment. For every stimulation frequency, different letters denote significant differences between different temperatures (ANOVA and Tukey HSD post-hoc Test;  $\alpha=0.05$ ,  $P < 0.05$ ).

**Fig. 7.** Peak characteristics calculated from EAG recordings of *S. exigua*, *C. cautella* and *P. gossypiella* at room temperatures of 18, 23 and 28°C. Responses ( $\pm$  SD) were averaged from 10 replicates for each treatment. Repolarization time was calculated from the highest depolarization value to two-thirds of the peak amplitude. Different lower case letters denote significant differences within the same species (ANOVA and Tukey HSD post-hoc Test;  $\alpha=0.05$ ,  $P < 0.05$ ).

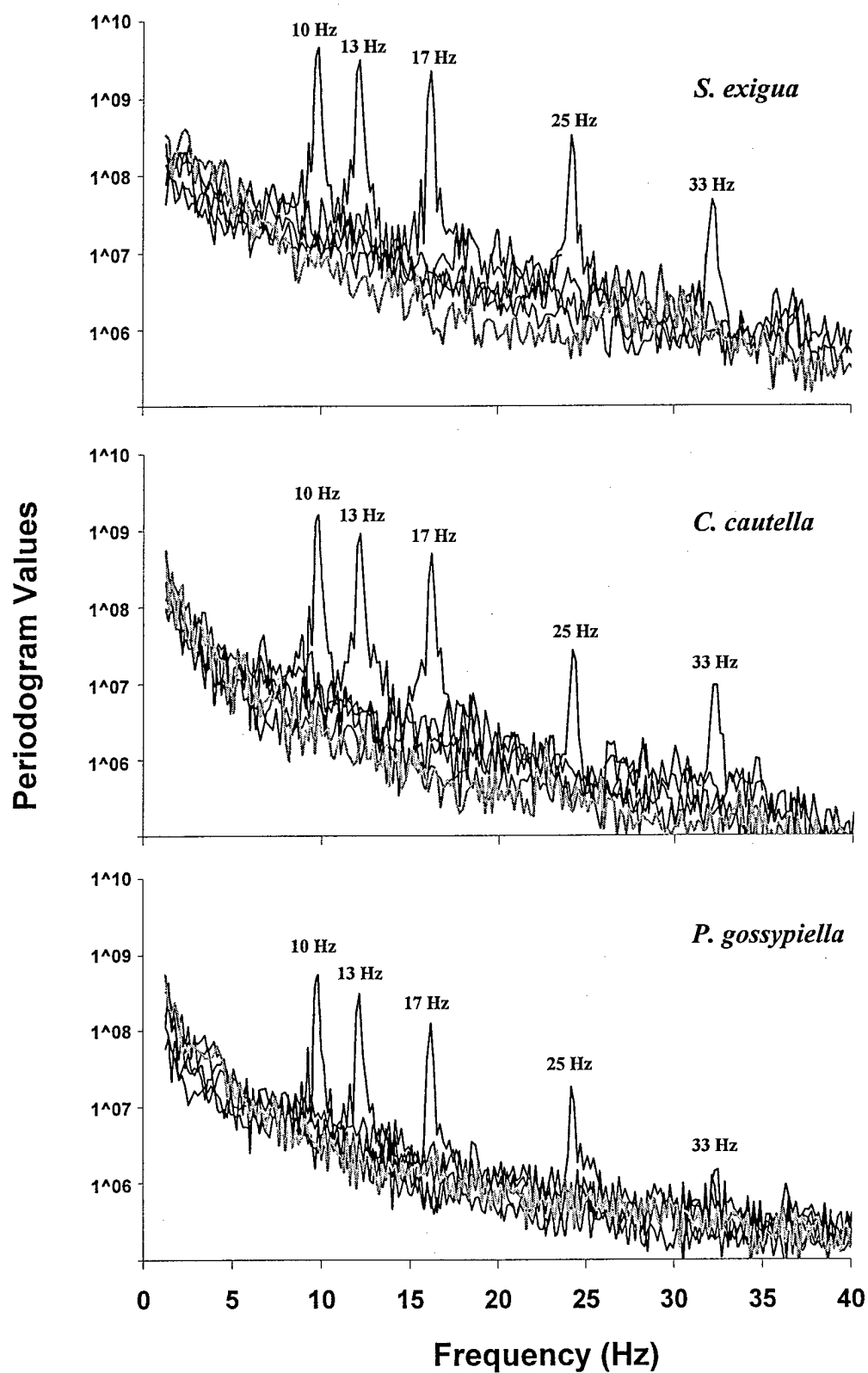


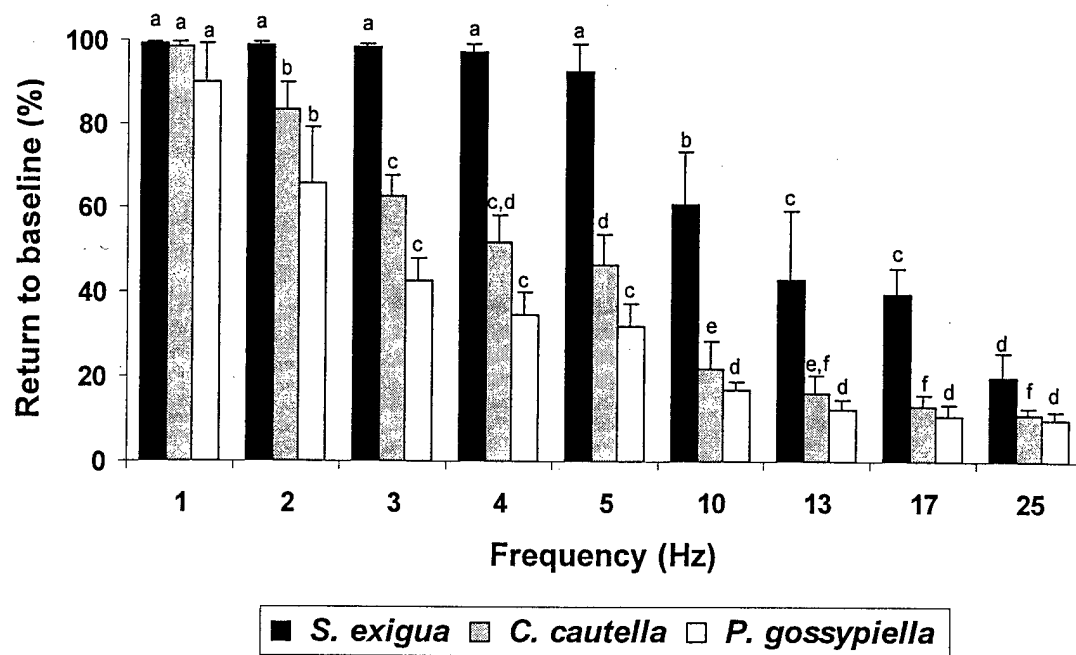
Bau et al. Fig. 1.



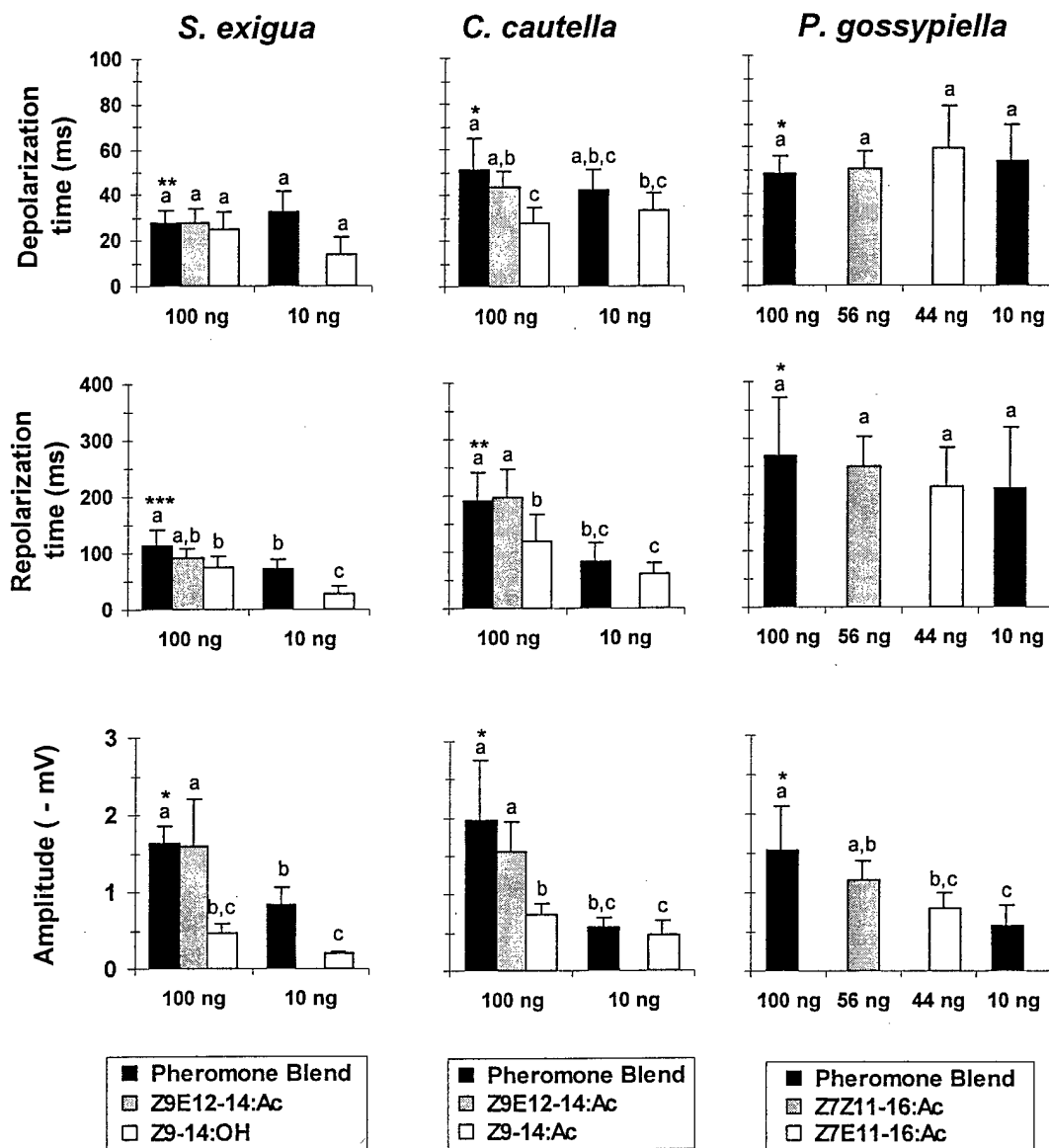


Bau et al. Fig. 3.

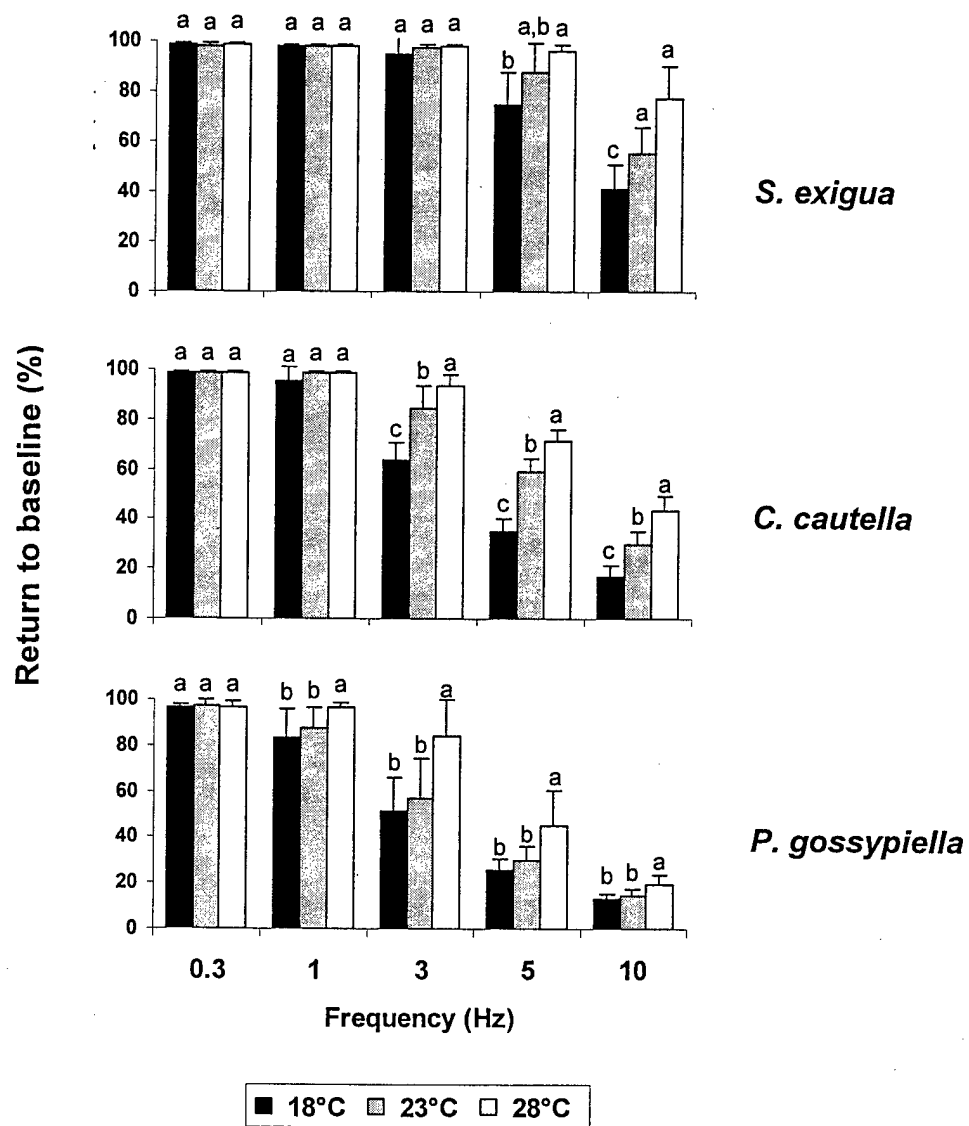




Bau et al. Fig. 5.



Bau et al. Fig. 6.



Bau et al. Fig. 7.

